ANNALS of Clinical and Translational Neurology
Open Access
AMERICAN NEUROLOGICAL ASSOCIATION

RESEARCH ARTICLE

Evaluation of serum and cerebrospinal fluid biomarkers after vaccination against SARS-CoV-2

Salvatore Iacono[1], Tommaso Piccoli[1], Paolo Aridon[1], Giuseppe Schirò[1], Valeria Blandino[1], Domenico Tarantino[1], Luisa Agnello[2], Marcello Ciaccio[2], Paolo Ragonese[1] & Giuseppe Salemi[1]

[1]Neurology Unit, Department of Biomedicine, Neuroscience and Advanced Diagnostics (BiND), University of Palermo, Palermo, Italy
[2]Department of Biomedicine, Neurosciences and Advanced Diagnostics, Institute of Clinical Biochemistry, Clinical Molecular Medicine and Clinical Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy

Correspondence
Paolo Ragonese, Section of Neurology, Department of Biomedicine, Neuroscience and Advanced Diagnostics (BiND), University of Palermo, Via Gaetano La Loggia, 1, Palermo, Italy. Tel: +39 0916555179; E-mail: paolo.ragonese@unipa.it

Received: 3 January 2023; Revised: 11 April 2023; Accepted: 15 April 2023

***Annals of Clinical and Translational Neurology* 2023; 10(6): 1025–1034**

doi: 10.1002/acn3.51785

Abstract

Objective: Vaccines are a major achievement of science, and new vaccines against SARS-CoV-2 are protecting the entire population from a life-threatening infection. Although several neurological complications or worsening of pre-existing neurological conditions after vaccination have been observed, whether a biological plausibility exist between new vaccines against-SARS-CoV-2 and neurological consequences is unclear. The aim of this study is to evaluate whether vaccines against SARS-CoV-2 induce systemic or cerebrospinal fluid alterations in patients with neurological disorders. **Methods**: Patients who underwent lumbar puncture (LP) between February 2021 and October 2022 were enrolled. Serum C-reactive protein (CRP), neutrophil to lymphocyte ratio (NLR), cerebrospinal fluid total protein content (CSF-TPc), glucose CSF/serum ratio, number of CSF cells per cubic millimeter, and CSF neurofilament light chain (CSF-NfL) were compared between unvaccinated and vaccinated patients. **Results**: A total of 110 patients were included and fitted into three groups according firstly to vaccination status (vaccinated and unvaccinated) and then to time from last dose of vaccine to LP (within or after 3 months). TPc, CSF/S_{Glu} ratio, number of cells per cubic millimeter, CSF-NfL, CRP, and NLR were not different between groups (all $p > 0.05$), and also, they did not differ neither according to age nor diagnosis. No relevant differences between groups were also noticed when the at-risk time window was set to 6 weeks. **Interpretation**: No signs of neuroinflammation, axonal loss and systemic inflammation were found in patients with neurological disorders after anti-SARS-CoV-2 vaccination compared with unvaccinated ones.

Introduction

In 2020, SARS-CoV-2 infection underlying coronavirus disease 19 (COVID-19) rapidly became a worldwide health emergency due to its elevated infecting capacity, morbidity, and mortality.[1] However, in early 2021 COVID-19 vaccines have brought a hope to fight against this infection. Also giving the detrimental effect of COVID-19 in patients with neurological disease, vaccination against SARS-CoV-2 became essential in these patients.[2] To date, many vaccines against-SARS-CoV-2 are approved, some of these are based on nucleoside-modified mRNA encoding for spike (S) lipoprotein (e.g., BNT162b2, mRNA-1273) and other viral-vector based containing the genetic information to encoding S protein (e.g., ChAdOx1-S, Ad26.COV2-S).[3–6] Once introduced, genetic material helps in making SARS-CoV-2 S protein which is recognizes by immunity cells producing a defensive immune reaction. It has been reported that all kinds of vaccines are associated with several neurological complications such as Guillain–Barré syndrome (GBS) and acute disseminated encephalomyelitis (ADEM).[7] As a part of causality criteria, the biological plausibility of these post-vaccination complication had been ascribed to molecular mimicry, direct neurotoxicity, and aberrant immune reactions.[7] In this scenario, it could be

reasonable to wonder whether new vaccines against SARS-CoV-2 may induce systemic or neuroinflammatory response representing the epiphenomenon of a biological plausibility, underlying the neurologic post-vaccination complications. On the contrary, it could be reasonable to wonder whether COVID-19 vaccines may worsen a pre-existing neurological disease. Apart from the biological plausibility, other relevant issue for the establishment of causality is the temporal relationship between vaccination and adverse events following immunization (AEFI).[8] Many authors consider a time of 6 weeks as at-risk window for complication after SARS-CoV-2 vaccination based on the at-risk window for GBS after influenza A vaccination.[9–11] However, a precise at-risk period after the occurrence of an external pathogenetic noxa (e.g., vaccination or viral infection) is not established, and it is extremely variable in the reported cases. Thus, it could be possible to speculate whether COVID-19 vaccines containing SARS-CoV-2 epitopes are able to induce subclinical and subtle alterations in cerebrospinal fluid (CSF) and serum inflammation parameters temporally preceding a clinical manifestation. We aim to explore the occurrence of subtle systemic or nervous system inflammatory alterations in a cohort of patients with neurologic disorders who underwent to diagnostic lumbar puncture (LP) by exploring CSF and systemic inflammation biomarkers (i.e., total protein content, CSF glucose, serum to CSF glucose ratio, number of CSF cells and serum C reactive protein and neutrophil to lymphocyte ratio, respectively) and comparing patients who underwent LP after COVID-19 vaccination and those who did not receive vaccination.

Materials and Methods

Study design and patients' collection

We performed a retrospective observational study to explore whether vaccines against SARS-CoV-2 induced alteration of CSF and serum inflammation parameters in patients with multiple sclerosis (MS), neurodegenerative diseases (Alzheimer's disease, frontotemporal dementia, parkinsonism) and vascular dementia. We enrolled people attending the Neurology Unit of the University Hospital "Policlinico Paolo Giaccone," Palermo, Italy, who underwent diagnostic LP as a part of routinely diagnostic work up between February 1, 2021 and October 31, 2022. Patients with age ≥18 years and signed informed consent form to perform LP and complete CSF collection were included.

Exclusion criteria

Age <18 years, not having a neurological disease after diagnostic work-up, lacking data (i.e., vaccination and/or laboratory), traumatic LP, systemic or CNS infection, diagnosis of Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy and CNS tumors were excluded from the final analysis. After the exclusion procedures, participants were divided into three groups: subjects who underwent LP within 3 months from vaccination, those who underwent LP after 3 months from vaccination and not vaccinated ones (Fig. 1).

Serum and cerebrospinal fluid biomarkers

For each participant serum glucose, white blood cells count (WBC) with neutrophils and lymphocytes count and serum C reactive protein (CRP) were collected. We calculated the neutrophil to lymphocyte ratio (NLR) from the ratio between neutrophils and lymphocytes. CRP is a multifunctional component of the innate immune system and its production is enhanced in the acute-phase response to inflammation which can be activated by tissue damage, infections, and vaccination. The differential count of WBC is another marker of systemic inflammation since this condition is characterized by neutrophilia and lymphocytopenia making thus the NLR a more reliable biomarker of systemic inflammation. We tested serum CRP, WBC, and NLR as biomarkers of systemic inflammation according to existing evidence.[12–14] CSF parameters analyzed were CSF total protein content (CSF-TPc), CSF glucose levels, CSF to serum glucose ratio (CSF/S_{Glu}), number of CSF cells per cubic millimeter (CSF cells/mm^3), and CSF neurofilament light chain (CSF-NfL). CSF-TPc represents a screening test to assess the blood–brain barrier function whether inflammation of the CNS is suspected. As well, number of CSF cells/mm^3, LCR glucose levels, and CSF/S_{Glu} ratio are increased and reduced, respectively, during CNS inflammation and they are a part of the routinely LCR laboratory testing.[15,16] CSF-NfL levels have been proportionally correlated to the degree of axonal damage in several neurologic disorders including inflammatory and degenerative CNS disease.[17,18] Also, NfL levels may increase several years before a disease onset being thus and early biomarker of CNS damage.[19,20] We tested for each participants CSF-NfL levels as biomarker of vaccine-induced CNS damage.

Sample collection and laboratory processing

Lumbar puncture was performed using a standardized technique with a 22 or 24 G "atraumatic" spinal needle and a sitting position.[21] CSF was collected using polypropylene tubes. CSF samples were analyzed within 2 h from collection as a part of the diagnostic work-up at the Central Laboratory of the University Hospital "Paolo Giaccone," Palermo, Italy. Serum and CSF collection and

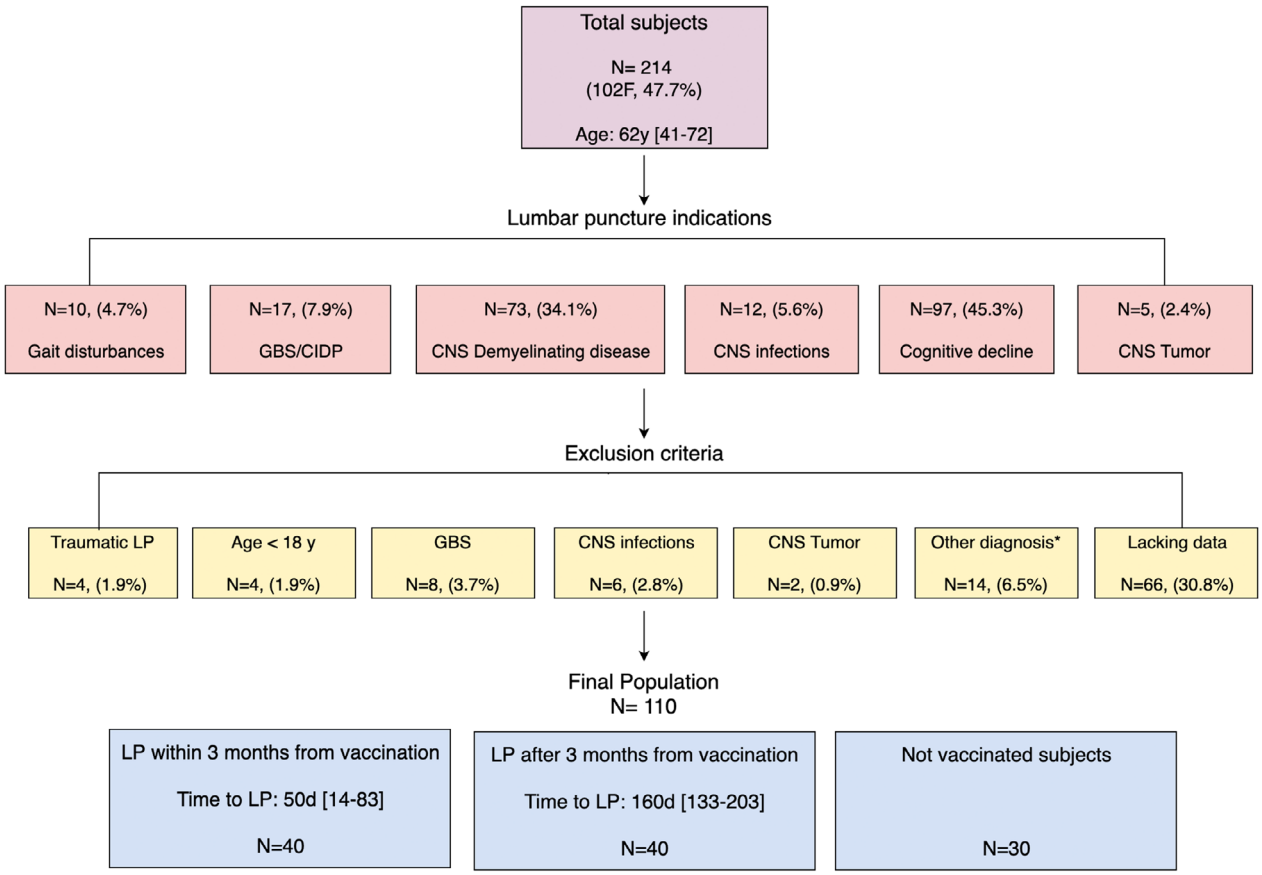

Figure 1. Flow chart showing the total number of enrolled participants, lumbar puncture indications and the selection of the final population according to exclusion and inclusion criteria of the study. *Not having a neurological disorder including healthy subjects after the diagnostic work-up. Interquartile ranges are reported within squared brackets. CIDP, chronic inflammatory demyelinating polyneuropathy; CNS, central nervous system; d, days; F, female; GBS, Guillain–Barré syndrome; LP, lumbar puncture; y, years.

analyses were carried out at the same day. CSF-NfL levels were analyzed using the commercially available ELISA kit UmanDiagnostics NF-light® assay, (Umeå, Sweden). Blood samples were collected at the enrolment in K3-EDTA tubes (Greiner Bio-One) from all the participants in fasting state. WBC with neutrophils and lymphocytes counts was assessed within 3 h by flow cytometry (Beckman coulter, Pasadena, CA, USA), according to the manufacturer's instructions, while CRP was assayed by an immunoturbidimetric method using the ARCHITECT cSystems (MULTIGENT CRP Vario assay). The normal values for each parameters were established according to the normal ranges provided from our laboratory: WBC (2 to 11 10^3 cells/μL), neutrophils (2 to 8 10^3 cells/μL), lymphocytes (1 to 5 10^3 cells/μL), serum glucose (60 to 100 mg/dL), serum CRP (<5 mg/L), CSF-TPc (150 to 450 mg/dL), CSF glucose (40 to 60 mg/dL), CSF cells/mm^3 (<5), and CSF-NfL (<30 years [<380 pg/mL]; 30 to 40 years [<560 pg/mL]; 40 to 60 years [<890 pg/mL]; >60 years [<1850 pg/mL]). A CSF/S_{Glu} from 0.3 to 0.5 were considered as within normal range.[22] The normal NLR cutoff was set from 0.78 to 3.53.[23]

Statistical analysis

Shapiro–Wilks test was used to check normality. Quantitative variables were reported as median and interquartile ranges (IQR) within squared brackets or as mean and standard deviation (SD) as appropriate. Qualitative variables were reported as number and relative percentage. Association between qualitative variables was estimated by using Pearson's chi-squared test or Fisher's exact test while quantitative data were compared among groups through Mann and Whitney or Kruskal–Wallis tests, as appropriate. Analysis of variance (ANOVA) was employed to analyze continuous data with normal distribution. Spearman's correlation analyses (rs) were employed to estimate the association between quantitative variables.

The association between the variables considered as indicators of CSF inflammatory markers was performed calculating time from vaccination to lumbar puncture, either as continuous variable or as categorizing it, as above described. Statistical analyses were performed using the SPSS package (IBM Corp. Released 2019. IBM SPSS Statistics for MacOS, Version 26.0. Armonk, NY: IBM Corp). The statistical significance has been set as two tailed with $p < 0.05$.

Results

Shapiro–Wilk test was performed to assess normality, and it showed a not-normal distribution for overall continuous variables (all $p < 0.05$) with exception of CSF/S_{Glu} ratio ($p = 0.11$). A total of 214 patients were included in the study, and a total of 100 and 10 were included in the final analysis according to exclusion criteria (Fig. 1). Sex ($p = 0.82$), age ($p = 0.07$), and final diagnosis (all $p > 0.05$) were similar among groups. Out of 18 participants, $n = 11$ received only one vaccine's dose (13.8%), while $n = 32$ (40%), $n = 35$ (43.8), and $n = 2$ (2.5%) received two, three, and four doses, respectively (Table 1). A statistically significant higher percentage of participants vaccinated within 3 months before LP received one vaccine's dose compared to those vaccinated at least 3 months before LP ($p = 0.003$) while these received more commonly three doses ($p = 0.04$). Participants who underwent LP after at least 3 months from vaccination showed a higher median number of doses received ($p = 0.04$; Table 1). In the Table 1 are summarized the demographic features, final diagnosis and vaccination information (i.e., type of vaccine and number of doses, time from vaccination to LP) of the participants included in the final analysis.

Serum analysis

Unvaccinated patients showed higher WBC, neutrophils, and lymphocytes compared with vaccinates ones (Table 2). CRP serum levels and NLR were within normal limits in all the participants, and they were similar among groups (all $p > 0.05$; Table 2). Serum CRP median levels were not different according to vaccination status after

Table 1. Demographic features, diagnosis, and vaccination information of the subjects included in the final analysis according to vaccination status and time lasted between vaccination and lumbar puncture.

	Overall *N* = 110 *N* (%)	LP ≤3 months *N* = 40 *N* (%)	LP >3 months *N* = 40 *N* (%)	Not vaccinated *N* = 30 *N* (%)	*p**
Male	50 (45.5)	18 (45)	17 (42.5)	15 (50)	0.82
Age median [IQR]	64 [47–72]	68 [53–73]	69 [46–74]	59 [43–65]	0.07
Age > 60 years	66 (59.8)	26 (65)	27 (67.5)	13 (43.3)	0.09
Inflammatory disease					
Multiple sclerosis	40 (36.4)	12 (30)	13 (32.5)	15 (50)	0.19
Not inflammatory disease					
Alzheimer's disease	39 (35.5)	15 (37.5)	18 (45)	6 (20)	0.09
Frontotemporal dementia	9 (8.2)	4 (10)	3 (7.5)	2 (6.7)	0.86
Parkinsonism	11 (10)	5 (12.5)	2 (5)	4 (13.3)	0.42
Vascular dementia	11 (10)	4 (10)	4 (10)	3 (10)	1
Vaccine	80 (72.7)	40 (100)	40 (100)	–	
BNT162b2 (Pfizer-BioNTech)	53 (66.3)	29 (72.5)	24 (60)	–	0.24
mRNA-1273 (Moderna)	22 (27.5)	8 (20)	14 (35)	–	0.13
ChAdOx1 (AstraZeneca)	5 (6.3)	3 (7.5)	2 (5)	–	0.64
Doses information					
One dose	11 (13.8)	10 (25)	1 (2.5)	–	0.003
Two doses	32 (40)	15 (37.5)	17 (42.5)	–	0.65
Three doses	35 (43.8)	13 (32.5)	22 (55)	–	0.04
Four doses	2 (2.5)	2 (5)	0	–	0.5
Number of doses median [IQR]	2 [2–3]	2 [1–3]	3 [2–3]	–	0.04
Time to LP[1], days, median [IQR]	108, [48–163]	50, [14–83]	160, [133–203]	–	–

IQR, interquartile range; LP, lumbar puncture; *N*, number.
[1]Time in days between vaccination and lumbar puncture.
**p* values for trend.

Table 2. Comparison analyses of cerebrospinal fluid and serum parameters between subjects who underwent lumbar puncture within 3 months, after 3 months from vaccination and not vaccinated ones.

	Overall $N = 110$	LP ≤3 months $N = 40$	LP >3 months $N = 40$	Not vaccinated $N = 30$	p*
Serum parameters					
Serum CRP, median [IQR], mg/L	0.98 [0.6–2]	0.9 [0.6–1.5]	0.83 [0.6–1.5]	1.2 [0.6–3.4]	0.3
WBC, median [IQR], 10^3 cells/μL	6.9 [5.6–8.2]	6.4 [5.6–9.3]	6.8 [5.3–8]	8.3 [6.5–10.1]	0.015[ʃ]
Neutrophils, median [IQR], 10^3 cells/μL	4.5 [3.3–5.5]	4 [3.4–5.2]	4.4 [3.3–5.2]	5.2 [3.8–6.5]	0.046[Δ]
Lymphocytes, median [IQR], 10^3 cells/μL	1.7 [1.4–2.3]	1.7 [1.4–2.1]	1.5 [1.3–1.9]	2.0 [1.4–2.8]	0.016[ƒ]
NLR, median [IQR]	2.49 [1.9–3.3]	2.35 [1.8–3.4]	2.66 [2–3.2]	2.49 [1.9–3.3]	0.6
Serum glucose, median [IQR], mg/dL	91 [83–106]	91 [81–115]	91 [84–102]	91 [85–105]	0.9
CSF parameters					
TPc, median, [IQR], mg/L	350 [297–441]	318 [288–421]	379 [309–449]	367 [297–484]	0.3
Glucose, median, [IQR], mg/dL	60 [56–69]	61 [56–69]	60 [56–66]	60 [57–68]	0.9
CSF/S_{Glu} ratio, mean ± SD	0.68 ± 0.09	0.67 ± 0.09	0.68 ± 0.09	0.68 ± 0.1	0.9[†]
CSF cells/mm^3, median [IQR]	1.2 [0.8–3]	1.2 [0.8–3.2]	1.4 [0.7–4.7]	1.2 [0.9–2.8]	0.9
NfL, median [IQR], pg/mL	621 [373–1201]	854 [447–1267]	573 [378–1103]	542 [301–1130]	0.3

Kruskal–Wallis comparisons: [ʃ]LP ≤3 months vs. not vaccinated, $p = 0.02$; LP >3 months vs. not vaccinated, $p = 0.04$; LP ≤3 months vs. LP >3 months, $p = 1$; [Δ]LP ≤3 months vs. not vaccinated, $p = 0.02$; LP >3 months vs. not vaccinated, $p = 0.042$; LP ≤3 months vs. LP >3 months, $p = 0.74$. [ƒ]LP ≤3 months vs. not vaccinated, $p = 0.09$; LP >3 months vs. not vaccinated, $p = 0.016$; LP ≤3 months vs. LP >3 months, $p = 1$.
CRP, C reactive protein; CSF, cerebrospinal fluid; CSF/S_{Glu}, cerebrospinal fluid to serum glucose ratio; IQR, interquartile range; NfL: neurofilament light chain; NLR, neutrophil to lymphocyte ratio; SD, standard deviation; TPc, total protein content; WBC, white blood count.
*Kruskal–Wallis test with Bonferroni correction.
[†]One way ANOVA.

stratifying for age and diagnosis (all $p > 0.05$; Fig. 2A,C). Although median NLR was higher in the group of patients aged more than 60 years ($p = 0.026$; Fig. 2B), not significant differences were noticed between groups after stratifying for age and diagnosis (all $p > 0.05$; Fig. 2B,D). Correlation analyses did not show a statistically significant correlation between serum inflammation parameters neither with time from last dose to LP (CRP: $rs = -0.04$; $p = 0.71$ and NLR: $r = 0.07$, $p = 0.56$) nor with number of doses received (CRP: $rs = 0.18$; $p = 0.13$ and NLR: $r = -0.05$, $p = 0.97$). Age was positively correlated with serum glucose ($rs = 0.3$; $p = 0.003$).

CSF analysis

TPc was not different between groups (Table 2), and it did not differ neither after stratifying for age nor for diagnosis (all $p > 0.05$; Fig. 3A,B). CSF/S_{Glu} ratio did not differ between groups (Table 2) and although it was higher in patients aged ≤60 years old (0.7 ± 0.1 vs. 0.66 ± 0.09; $p = 0.04$), not group effect was detected after stratifying for age and diagnosis (all $p > 0.05$; Fig. 3C,D). Correlations did not show significant association between time from last dose to LP, CSF-TPc ($rs = 0.22$; $p = 0.06$) and CSF/S_{Glu} ratio ($rs = 0.07$; $p = 0.56$) as well as no correlation emerged between number of doses received and TPc ($rs = 0.21$; $p = 0.06$) or CSF/S_{Glu} ratio ($rs = -0.06$; $p = 0.66$). Csf-NfL levels were higher in patients aged more than 60 years (465 pg/mL [283–1063] vs. 712 pg/mL [499–1401]; $p = 0.004$; Fig. 4A) and in those with not inflammatory diseases (694 pg/mL [461–1345] vs. 496 pg/mL [309–1063]; $p = 0.025$; Fig. 4B) but a group effect was not found depending on vaccination status (all $p > 0.05$; Fig. 4A,B). The median number of CSF cells/mm^3 was higher in younger participants (3/mm^3 [1.2–8.3] vs. 1/mm^3 [0.6–2]; $p < 0.0001$; Fig. 4C) as well as in those with MS (3.7/mm^3 [1.6–8.8] vs. 1/mm^3 [0.6–1.6]; $p < 0.0001$; Fig. 4D) but it did not differ between groups according to vaccination status (all $p > 0.05$; Fig. 4A,D). Correlation analyses did not show a statistically significant correlation between CSF-NfL neither with time from last dose to LP ($rs = -0.04$, $p = 0.72$) nor with number of doses received ($rs = -0.004$; $p = 0.97$). Number of CSF cells/mm^3 did not correlate with number of doses received ($rs = 0.01$; $p = 0.9$) nor with time from last dose to LP ($rs = 0.03$; $p = 0.77$).

Comorbidity and medications

A total of seventy-one patients were affected by a comorbidity without difference between groups ($p = 0.64$) except for dyslipidemia that was more common in patients who underwent LP within 3 months from vaccination ($p = 0.02$). As well, medications taken by participants at the time of vaccination did not differ overall between groups ($p = 0.89$) at the exception of antithrombotics drugs which were more commonly taken by participants who underwent LP after 3 months from

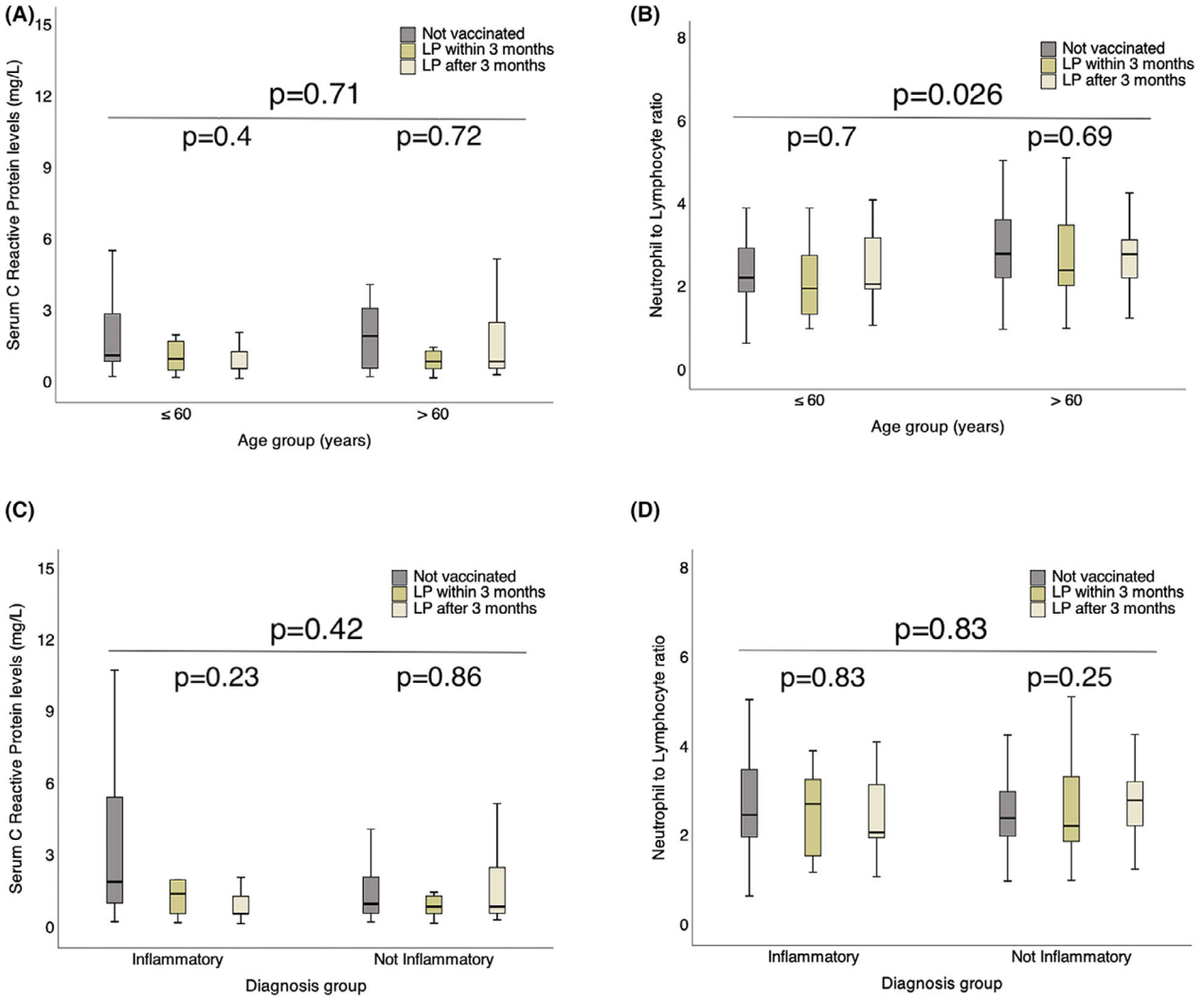

Figure 2. Group-matched boxplots for serum C-reactive protein levels and neutrophil to lymphocyte ratio stratifying for age (A and B) and diagnosis (C and D). The line within the boxes indicates the median while the boundaries of the boxes represent the 25th and 75th quartiles. The whiskers above and below the boxes correspond to the highest and lowest values. Mann and Whitney and Kruskal–Wallis tests were used for all comparison.

vaccination ($p = 0.006$). All the comorbidities and medications taken by the participants included in the final analysis are listed in Table S1.

Discussion

In this study, we evaluated whether COVID-19 vaccines induced serum and CSF alterations in a cohort of patients with MS, vascular dementia, and neurodegenerative diseases to evaluate whether vaccines against-SARS-CoV-2 are able to induce subtle systemic and CNS inflammation. Indeed, several case report or case series have been published reporting neurological inflammatory complications after vaccination against-SARS-CoV-2 such as GBS, ADEM, transverse myelitis, optic neuritis, and active CNS demyelination.[24–27] Also, Stastna et. al reported a mild increase in the relapse incidence after anti-SARS-CoV-2 vaccination period in patients with MS and neuromyelitis optica spectrum disorder.[27,28] Firstly, we did not find any changes of WBC, neutrophils, and lymphocytes in vaccinated patients compared to not vaccinated ones (Table 2) suggesting an absence of vaccine-induced systemic inflammation. Salvagno et al. found that higher serum CRP levels are associated with a higher response to BNT162b2 booster administration[29]; however, we further did not find any correlation between time from vaccination, number of doses received, and CRP levels neither after stratifying for age nor for diagnosis (Fig. 2A,C). Although

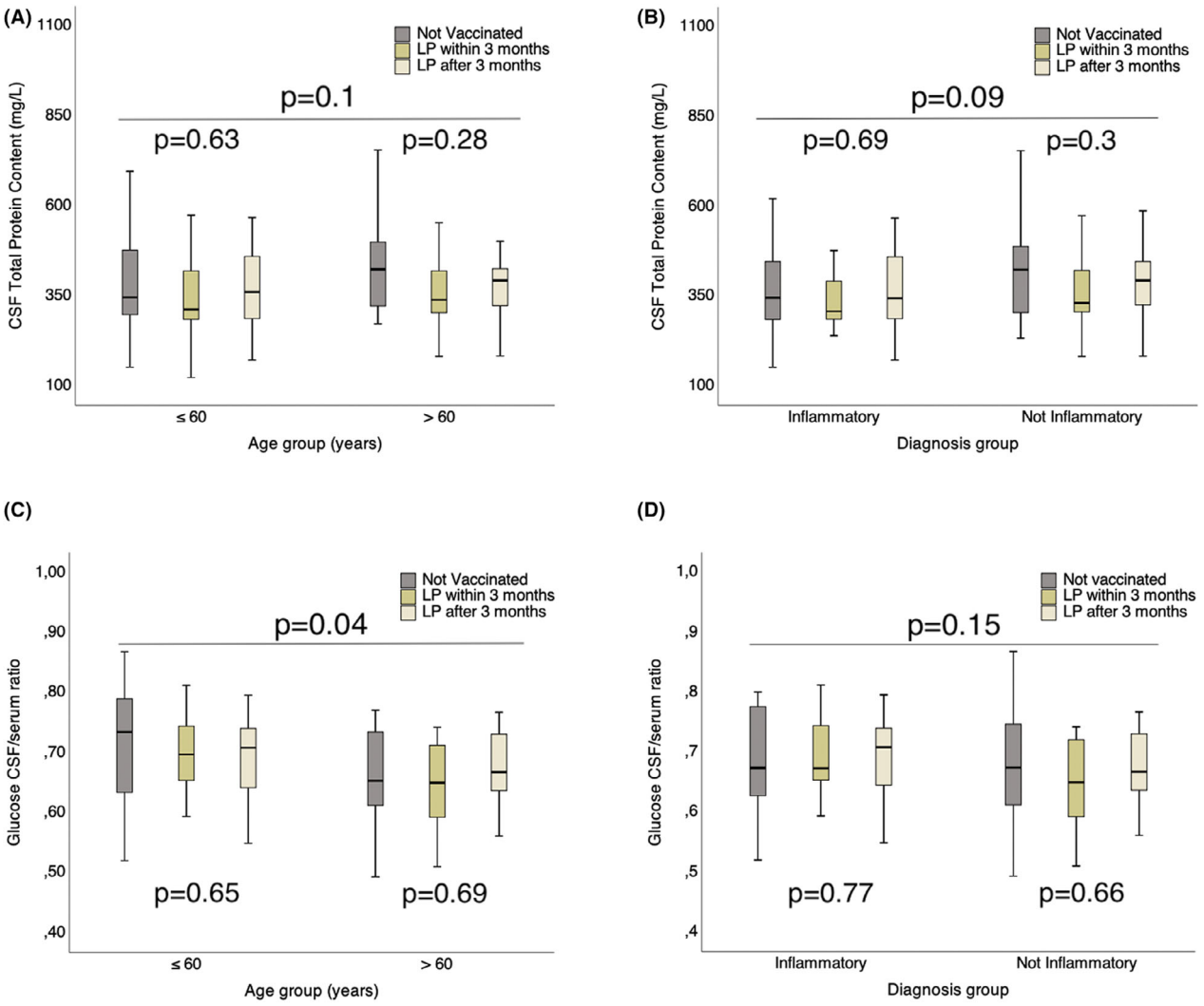

Figure 3. Group-matched boxplots for CSF total protein content and glucose CSF/serum ratio stratifying for age (A and C) and diagnosis (B and D). The line within the boxes indicates the median while the boundaries of the boxes represent the 25th and 75th quartiles. The whiskers above and below the boxes correspond to the highest and lowest values. Mann and Whitney and Kruskal–Wallis tests were used for all comparison.

NLR was increased in older participants (Fig. 2B), we found no association between groups as well as after subgrouping for age and diagnosis (Fig. 2B,D). This result is consistent with a previous report that showed no NLR alterations following BNT162b2 administration.[30] In our study, we found no elevation of CSF-TPc in vaccinated patients compared to others (Fig. 3A,B) as well as no correlations between time from vaccination to LP and number of doses received and TPc were found. CSF/S_{Glu} ratio was lower in patients aged more than 60 years, and it could be due to increased serum glucose levels in older participants with subsequently reduced CSF/S_{Glu} ratio. However, CSF/S_{Glu} ratio was almost unchanged among groups according to age and diagnosis (Fig. 3A,B). Of interest, we found that number of CSF cells/mm^3 was higher in patients with MS in a not-dependent manner to vaccination (Fig. 4C,D); however, a higher number of CSF cells per cubic millimeter in patients with MS is actually expected.[31] We found higher CSF-NfL levels in patients with neurodegenerative/vascular diseases compared with MS patients and in those aged more than 60 years, but no vaccination effect was found neither stratifying for age nor for diagnosis (Fig. 4A,B). The higher CSF-NfL levels in patients with neurodegenerative/vascular diseases comparing to others were, however, expected according to literature.[32,33] Overall, CSF-TPc, number of CSF cell per cubic millimeter and CSF-NfL levels were not temporally correlated with vaccination

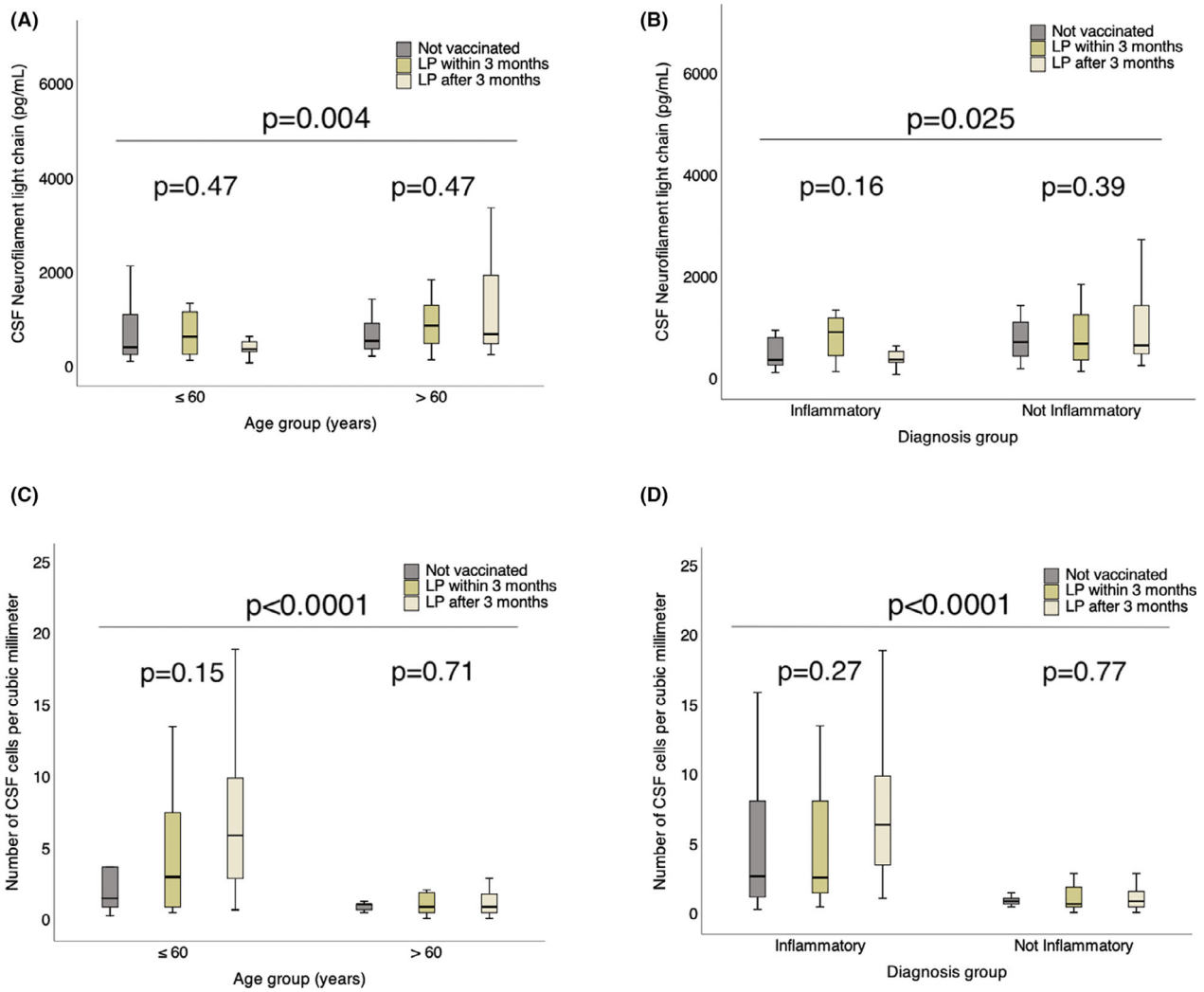

Figure 4. Group-matched boxplots for CSF neurofilament light chain levels and number of CSF cells per cubic millimeter stratifying for age (A and C) and diagnosis (B and D). The line within the boxes indicates the median while the boundaries of the boxes represent the 25th and 75th quartiles. The whiskers above and below the boxes correspond to the highest and lowest values. Mann and Whitney and Kruskal–Wallis tests were used for all comparison.

neither with number of doses received thus indicating a null effect of vaccination in serum and CSF inflammation as well as in axonal loss. Although the temporal criterion AEFI is estimated to be 6 weeks,[8] we considered a temporally window of 3 months from vaccination to fit participants into groups. Indeed, although the AEFIs related to vaccination usually occurs within 6 week from immunization, the more lately occurrence of subtle inflammatory alteration cannot be excluded, and it has not been explored so far. However, we conducted a preliminary analysis comparing CSF parameters, serum CRP and NLR between participants who underwent LP within 6 weeks from vaccination and other (after 6 weeks and controls) but even in this case no relevant differences were noticed (Table S2). Finally, we did not observe any significant difference between the cohorts with respect to the comorbidities or the medications used by included individuals, possibly modifying vaccine response (Table S1).

The medium (within 3 months) and long-term (after 3 months) evaluation of CSF and serum inflammation parameters is a strength of our study which is not limited only to the short-term evaluation (i.e., within 6 months after vaccination). Other strength of this study is the employing of strictly exclusion criteria that limited the bias due to factor not related with vaccination. Some limitations should be considered when interpreting our data: (1) the small number of sample size may have reduced the power of some statistical analyses; (2) due to the

small number of participants immunized with viral vectors-based vaccines, we could not perform sub-group analysis according to vaccine type; (3) local and systemic AEs following vaccination were not collected and the relationship between CSF or serum changes with AEs has not been performed. To the best of our knowledge, this is the first report showing that immunization against SARS-CoV-2 is not associated with alteration of CSF-TPc, CSF-glucose, CSF/S_{Glu} ratio, CSf-NfL, serum CRP, and NLR in patients with inflammatory and not inflammatory neurological diseases. We may conclude that new vaccines against SARS-CoV-2 are not associated with serum inflammation, CSF changes and axonal damage in patients with MS, neurodegenerative diseases, and vascular dementia.

Author Contributions

S.I. and P.R. conceptualized and designed the study. S.I., T.P., P.A., G.Sch., V.B., and D.T. collected the data. M.C. and L.A. carried out the laboratory analyses. S.I., G.Sa., and P.R. analyzed and interpreted the data. S.I. drafted the manuscript. T.P., P.A., P.R., and G.Sa. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding Information

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Policlinico Paolo Giaccone Palermo I. The study was performed in the settings of standard clinical practice whereby a specific informed consent form for lumbar puncture and CSF analyses was submitted to each patient as part of the routine work out.

Patients Consent Statement

All the patients gave their consent to the study purpose.

Data Availability Statement

Data are available from the corresponding author upon a reasonable request.

References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
2. Marsh E, Kornberg M, Kessler K, et al. COVID-19 and vaccination in the setting of neurologic disease: an emerging issue in neurology. Neurology. 2021;97:720-728. doi:10.1212/WNL.0000000000012578
3. Bellino S. COVID-19 vaccines approved in the European Union: current evidence and perspectives. Expert Rev Vaccines. 2021;20(10):1195-1199. doi:10.1080/14760584.2021.1962304
4. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383(27):2603-2615. doi:10.1056/NEJMOA2034577
5. Baden LR, el Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384(5):403-416. doi:10.1056/NEJMOA2035389
6. Voysey M, Clemens SA, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021;397(10269):99-111. doi:10.1016/S0140-6736(20)32661-1
7. Piyasirisilp S, Hemachudha T. Neurological adverse events associated with vaccination. Curr Opin Neurol. 2002;15(3):333-338. doi:10.1097/00019052-200206000-00018
8. WHO. Causality assessment of an adverse event following immunization (AEFI) 2018 user manual for the revised who classification second edition. WHO; 2018. Accessed December 16, 2022 http://apps.who.int/bookorders
9. Keh RYS, Scanlon S, Datta-Nemdharry P, et al. COVID-19 vaccination and Guillain-Barré syndrome: analyses using the national immunoglobulin database. Brain. 2022;18:739-748. doi:10.1093/BRAIN/AWAC067
10. Frontera JA, Tamborska AA, Doheim MF, et al. Neurological events reported after COVID-19 vaccines: an analysis of VAERS. Ann Neurol. 2022;91(6):756-771. doi:10.1002/ANA.26339
11. Salmon DA, Proschan M, Forshee R, et al. Association between Guillain-Barré syndrome and influenza a (H1N1) 2009 monovalent inactivated vaccines in the USA: a meta-analysis. Lancet. 2013;381(9876):1461-1468. doi:10.1016/S0140-6736(12)62189-8
12. Song M, Graubard BI, Rabkin CS, Engels EA. Neutrophil-to-lymphocyte ratio and mortality in the United States general population. Sci Rep. 2021;11(1):464. doi:10.1038/S41598-020-79431-7
13. Olsson A, Gustavsen S, Gisselø Lauridsen K, et al. Neutrophil-to-lymphocyte ratio and CRP as biomarkers in multiple sclerosis: a systematic review. Acta Neurol Scand. 2021;143(6):577-586. doi:10.1111/ANE.13401

14. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018;9:754. doi:10.3389/FIMMU.2018.00754
15. Thompson EJ, Keir G. Laboratory investigation of cerebrospinal fluid proteins. Ann Clin Biochem. 1990;27 (Pt 5):425-435. doi:10.1177/000456329002700503
16. Seehusen DA, Reeves MM, Fomin DA. Cerebrospinal fluid analysis. Am Fam Physician. 2003;68(6):1103-1108. Accessed April 12, 2022. https://pubmed.ncbi.nlm.nih.gov/14524396/
17. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018;14(10):577-589. doi:10.1038/S41582-018-0058-Z
18. Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019;90(8):870-881. doi:10.1136/JNNP-2018-320106
19. Bjornevik K, Cortese M, Healy BC, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science. 2022;375 (6578):296-301. doi:10.1126/SCIENCE.ABJ8222
20. Bjornevik K, Munger KL, Cortese M, et al. Serum neurofilament light chain levels in patients with Presymptomatic multiple sclerosis. JAMA Neurol. 2020;77 (1):58-64. doi:10.1001/JAMANEUROL.2019.3238
21. Doherty CM, Forbes RB. Diagnostic Lumbar Puncture. Ulster Med J. 2014;83(2):93-102. Accessed April 12, 2022. https://pubmed.ncbi.nlm.nih.gov/25075138/
22. Hegen H, Auer M, Deisenhammer F. Serum glucose adjusted cut-off values for normal cerebrospinal fluid/serum glucose ratio: implications for clinical practice. Clin Chem Lab Med. 2014;52(9):1335-1340. doi:10.1515/CCLM-2014-0077
23. Forget P, Khalifa C, Defour JP, Latinne D, van Pel MC, de Kock M. What is the normal value of the neutrophil-to-lymphocyte ratio? BMC Res Notes. 2017;10(1):1-4. doi:10.1186/S13104-016-2335-5
24. Patone M, Handunnetthi L, Saatci D, et al. Neurological complications after first dose of COVID-19 vaccines and SARS-CoV-2 infection. Nat Med. 2021;27(12):2144-2153. doi:10.1038/S41591-021-01556-7
25. Lahoz Fernandez PE, Miranda Pereira J, Fonseca Risso I, et al. Guillain-Barre syndrome following COVID-19 vaccines: a scoping review. Acta Neurol Scand. 2022;145 (4):393-398. doi:10.1111/ANE.13575
26. Arnao V, Maimone MB, Perini V, Lo Giudice G, Cottone S. Bilateral optic neuritis after COVID vaccination. Neurol Sci. 2022;43(5):2965-2966. doi:10.1007/S10072-021-05832-9
27. Khayat-Khoei M, Bhattacharyya S, Katz J, et al. COVID-19 mRNA vaccination leading to CNS inflammation: a case series. J Neurol. 2022;269(3):1093-1106. doi:10.1007/S00415-021-10780-7
28. Stastna D, Menkyova I, Drahota J, et al. To be or not to be vaccinated: the risk of MS or NMOSD relapse after COVID-19 vaccination and infection. Mult Scler Relat Disord. 2022;65:65. doi:10.1016/J.MSARD.2022.104014
29. Salvagno GL, Henry BM, Pighi L, de Nitto S, Lippi G. Serum C reactive protein predicts humoral response after BNT162b2 booster administration. J Infect. 2022;85(1): e24-e25. doi:10.1016/J.JINF.2022.04.015
30. Mediu R, Rama A, Puca E. Evaluation of neutrophil-to-lymphocyte ratio and immune response in patients vaccinated with Pfizer-Biontech vaccine. J Infect Dev Ctries. 2022;16(5):745-751. doi:10.3855/JIDC.16310
31. Deisenhammer F, Zetterberg H, Fitzner B, Zettl UK. The cerebrospinal fluid in multiple sclerosis. Front Immunol. 2019;10:726. doi:10.3389/FIMMU.2019.00726
32. Petzold A, Keir G, Warren J, Fox N, Rossor MN. A systematic review and meta-analysis of CSF neurofilament protein levels as biomarkers in dementia. Neurodegener Dis. 2007;4(2–3):185-194. doi:10.1159/000101843
33. Skillbäck T, Farahmand B, Bartlett JW, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology. 2014;83 (21):1945-1953. doi:10.1212/WNL.0000000000001015

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1.

